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SYNTHESIS OF THIOPHENES AND THIENO[3,2-c]PYRAN-4-ONES AS ANTILEISHMANIAL AND ANTIFUNGAL AGENTS#

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Abstract: A series of highly functionalized thiophene (**2**) and thieno[3,2-c]pyran-4-one(**4**) derivatives have been synthesized and evaluated for their antileishmanial and antifungal activities.

Introduction: Increasing pressure due to limited number of drugs in the clinical use for the treatment of leishmaniasis necessitated to develop new class of antileishmanial agents which could not only inhibit the growth of parasite but also boost the immune system of the host.

Except a single reference in the chemical literature[1], where potent antileishmanial activity of thiophene derivatives against *L. infantum LV9* is described, none has exploited the therapeutic potential of this nucleus even after more than a decade. Lack of proper recognition of therapeutic importance of thiophene (**2**) and thieno[3,2-c] pyran-4-one(**4**) derivatives prompted us to explore their potential as antileishmanial agents.

All the synthesized compounds **2** and **4** were evaluated for their antileishmanial activity against *L. donovani* promastigotes and some of them **2a**, **2f, 2k** and **21** demonstrated 95-100% growth inhibition of the parasites while **2b**, **2c**, **2h** and **4h** displayed 55-70% suppression at 25 μM concentration. The rest of the compounds were found either inactive or poorly active.

Thiophene nucleus possibly being a mimetic of imidazole or triazole binds to iron atom of the cytochrome P-450 haem and thereby displays potent antimycotic activity. This presumption led to explore the therapeutic potential of thiophene (**2**) and thieno[3,2-c]pyran-4-one (**4**) derivatives against opportunistic fungal pathogens[2]. Due to lack of clinically available vaccines or useful antisera for mycotic infections, numerous imidazole and triazole derivatives were developed as systemic antifungals[3] which disrupt the fungal membrane by inhibiting the action of lanosterol 14α-demethylase, a cytochrome P-450 enzyme.

All the synthesized compounds of the prototypes **2** and **4** were therefore screened for their antimycotic activity against five human pathogenic fungi, *Aspergillus fumigatus* (Af), *Candida albicans* (Ca), *Cryptococcus neoformans*(Cn), *Trichophyton mentagrophytes* (Tm) and *Sporothrix schenckii* (Ss) by two-

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fold serial dilution technique[4]. The activity of these compounds is expressed in terms of minimum inhibitory concentration (MIC) and compared with that of ketoconazole, a standard drug used. Among all the screened compounds only **2a** and **2n** demonstrated antimycotic activity against all the test fungi. Some of the compounds **2c, 2g** and **2k** demonstrated activity only against *T. mentagrophytes* with MIC of 50 μg/ml.

Synthesis: Various ketene dithioacetals (**1a-j**) used as synthon were synthesized by standard literature procedures[5-10]. These synthons on reaction with either ethyl mercaptoacetate or mercaptoacetic acid separately led to the formation of thiophene derivatives (**2a-o**) with different functionalities at specific position. A reaction of ketene dithioacetal (**1c**) and aryl methyl ketone under a basic condition[11] afforded 6-aryl-3-cyano-4-methylthio-2H-pyran-2-one (**3**) which on further reaction with ethyl mercaptoacetate provided 3-amino-6-aryl-2-carboethoxythieno[3,2-c]pyran-4-ones (**4**) (Scheme 1). The physicochemical data for representative compounds are shown in the reference section[12].

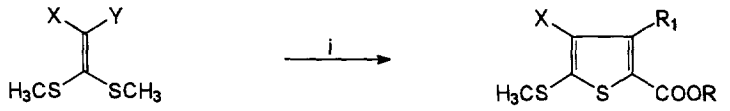
Biological Activity: The antileishmanial activity of thiophene (**2**) and thieno[3,2-c]pyran-4-one(**4**) was evaluated against promastigotes of *L. donovani* (UR6) *in vitro* as described below:

Promastigotes in the stationary phase of growth ($1x10^5$ parasite/ml) were inoculated into culture tubes containing Brain Heart Infusion-Agar (BHI-Agar) as solid part and Hank's Balance Salt Solution (HBSS) as liquid part. The test chemicals and pentamidine (25 μM, DMSO/PBS) were added to the above tubes respectively. The inhibitory effect of test compounds was compared with standard drug pentamidine. The whole operation was carried out asceptically in a U.V. chamber and tubes were inoculated for 5 days at 22°C. The antileishmanial activity of each compound was determined in triplicate by counting the number of live parasites per field microscopically and inhibition percentage was calculated and compared with standard drug pentamidine by using 'Z' statistic.

The *in vitro*. antifungal activity was assayed by two fold serial dilution technique[3] against five human pathogenic fungi *A. fumigatus, C. albicans, C. neoformans, S. schenckii* and *T. mentagrophytes*. Spores of the pathogens grown on Sabouraud's dextrose agar (SDA) slant incubated for 24-48 h (yeast) or 7 days (mycelial fungi) were suspended in Sabouraud's dextrose broth (SDB). The colony forming units (cfu) of the seeded broth were determined by dilution and plating technique and adjusted in the range 10^4-10^5 cfu/ml.

A solution of 0.2 ml of test compound (1mg/ml in DMSO) was added to 1.8 ml of the seeded broth to form the first dilution in assay tubes (size 13x100 mm). One ml of this solution was further diluted with 1 ml of seeded broth to make second dilution and so on till 10 such dilutions were obtained. A set of assay tubes with seeded broth and solvent were kept as control. The tubes were incubated in a BOD incubator at 28±1°C. The minimum inhibitory concentration (MIC) were recorded by visual observation after 24-48 h (yeast) and 72-96 h (mycelial fungi) incubation.

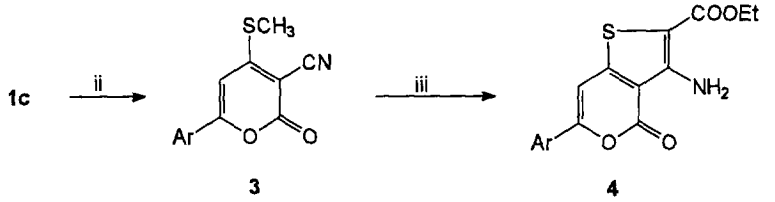
Scheme 1



1 2

1	X	Y
a	COOMe	COOMe
b	COOEt	COOEt
c	CN	COOEt
d	CN	CN
e	H	C_6H_5CO
f	H	4-FC_6H_4CO
g	H	4-ClC_6H_4CO
h	H	4-BrC_6H_4CO
i	H	4-$CH_3C_6H_4CO$
j	H	3-C_5H_4NCO

2	X	R	R_1
a	COOMe	Et	OH
b	COOEt	Et	OH
c	CN	Et	OH
d	CN	Et	NH_2
e	H	Et	C_6H_5
f	H	Et	4-FC_6H_4
g	H	H	4-FC_6H_4
h	H	Et	4-ClC_6H_4
i	H	H	4-ClC_6H_4
j	H	Et	4-BrC_6H_4
k	H	H	4-BrC_6H_4
l	H	Et	4-$CH_3C_6H_4$
m	H	H	4-$CH_3C_6H_4$
n	H	Et	3-pyridyl
o	H	H	3-pyridyl



3 4

4	Ar
a	4-FC_6H_4
b	4-ClC_6H_4
c	4-BrC_6H_4
d	3-$NO_2C_6H_4$
e	4-$NO_2C_6H_4$
f	4-$CH_3OC_6H_4$
g	3-pyridyl
h	2,4-Cl_2-5-FC_6H_2

Reagents/Conditions: i) $HSCH_2COOR$/DMF/KOH/25°C; ii) $ArCOCH_3$/DMF/KOH; iii) $HSCH_2COOEt$/KOH/MeOH/80°C

Table 1: *In vitro* antileishmanial and antifungal activities of thiophene(**2a-o**) and thieno[3,2-c]pyran-4- one (**4a-h**) derivatives

Compound No.	Antileishmanial activity (25 μM conc.) (% growth inhibition)	Antifungal activity Minimum Inhibitory Concentration in μg/ml against test fungi*				
		Af	Ca	Cn	Ss	Tm
2a	95	25	50	12.5	12.5	6.25
2b	63	>100	>100	>100	>100	>100
2c	60	>100	>100	>100	>100	50
2d	25	>100	>100	>100	>100	>100
2e	30	>100	>100	>100	>100	>100
2f	95	>100	>100	>100	>100	>100
2g	40	>100	>100	>100	>100	50
2h	55	>100	>100	>100	>100	>100
2i	30	>100	>100	>100	>100	>100
2j	20	>100	>100	>100	>100	>100
2k	100	>100	>100	>100	>100	50
2l	100	>100	>100	>100	>100	>100
2m	10	>100	>100	>100	>100	>100
2n	25	50	25	50	25	25
2o	25	>100	>100	>100	>100	>100
4a	30	>100	>100	>100	>100	>100
4b	24	>100	>100	>100	>100	>100
4c	8	>100	>100	>100	>100	>100
4d	28	>100	>100	>100	>100	>100
4e	20	>100	>100	>100	>100	>100
4f	10	>100	>100	>100	>100	>100
4g	15	>100	>100	>100	>100	>100
4h	70	>100	>100	>100	>100	>100
Pentamidine	100	--	--	--	--	--
Ketoconazole	--	0.7	0.1	0.3	0.1	25

*Af = *Aspergillus fumigatus*(AF-27), Ca = *Candida albicans*(SKF), Cn = *Cryptococcus neoformans*(CN-17), Ss = *Sporothrix schenckii*(SS-1), Tm = *Trichophyton mentagrophytes*(A-280)

All the synthesized compounds were evaluated for *in vitro* antileishmanial activity against *L. donovani* promastigotes. The activity of these compounds is shown in terms of % growth inhibition at the concentration of 25 μM. As it is evident from the Table 1, **2a**, **2f**, **2k** and **2l** are the most potent compounds displaying 95-100% growth inhibition of promastigotes of *L. donovani* while **2b**, **2c**, **2h** and **4h** suppressed the growth only 55-70%. Compounds showing below 50% of growth inhibition were treated as inactive. A structure-activity analysis revealed that most of the highly active compounds possess -COOEt group except **2k**, which has COOH substituent at position 2. The high order of activity may possibly be due to increased

lipophilicity due to presence of ester group. Nature of aryl substituent also potentiate the antileishmanial activity.

It is evident from the *in vitro* data of minimum inhibitory concentration (MIC) for compounds of prototype **2** against five selected fungi that the position and nature of the substituents at 3 and 4 are crucial. A compound (**2a**) with carbomethoxy group at position 4 and hydroxy group at 3 demonstrated significant antifungal activity against all the fungi. Increase in size of ester function at position 4 in **2b** from carbomethoxy to carboethoxy resulted in loss of efficacy. A further change of substituent in **2a** at position 4 from carbomethoxy to cyano in **2c** retained the activity only to *T. mentagrophytes* with MIC of 50 μg/ml. The acid derivatives **2g** and **2k** demonstrated significant activity only against *T. mentagrophytes* with MIC 50 μg/ml while their respective esters **2f** and **2j** were found inactive. The other acid derivatives **2i, 2m** and **2o** were inactive. This result revealed that the substituent at position 4 in **2** is responsible for expressing high order of activity. None of the compounds of the prototype **4** were found active.

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12. **Characterization data:**

2a: Yield: 82%; mp: 109°C; ^{1}H NMR($CDCl_3$) δ : 1.38 (t,3H,J=7.3 Hz,CH_3), 2.60 (s,3H,SCH_3), 3.96 (s,3H,OCH_3), 4.37 (q,2H,J=7.2 Hz,CH_2), 10.46(brs,1H,OH); IR(KBr): 1670 (CO), 1680 (CO), 3300 cm^{-1}(OH); MS m/z: [M^+] 276 (94), 244 (100); Anal. calcd. for $C_{10}H_{12}O_5S_2$: C,43.47; H,4.38. Found: C,43.72; H,4.41.

2h: Yield: 65%; mp: 64°C; ^{1}H NMR($CDCl_3$) δ : 1.24 (t,3H,J=7.3 Hz,CH_3), 2.60 (s,3H,SCH_3), 4.21 (q,2H,J=7.2 Hz,CH_2), 6.87(s,1H,CH), 7.49(d,2H,J=8.1 Hz, Ar-H), 7.61(d,2H,J=8.3Hz,Ar-H); IR(KBr): 1660 cm^{-1} (CO); MS m/z: [M^+] 312 (100); Anal. calcd. for $C_{14}H_{13}ClO_2S_2$: C,53.75; H,4.19. Found: C,53.89; H,4.29.

2n: Yield: 59%; mp: 66°C; ^{1}H NMR($CDCl_3$) δ : 1.24 (t,3H,J=7.4 Hz,CH_3), 2.62 (s,3H,SCH_3), 4.21 (q,2H,J=7.1 Hz,CH_2), 6.90(s,1H,CH), 7.30-7.34(t,1H,H-5'), 7.79(d,1H,J=7.9Hz,H-6'), 8.60(d,1H,J=8.0 Hz, H-4'), 8.64 (s,1H,H-2'); IR(KBr): 1650 cm^{-1} (CO); MS m/z: [M^+] 279 (100); Anal. calcd. for $C_{13}H_{13}NO_2S_2$: C,55.89; H,4.69; N,5.01. Found: C,55.93; H,4.78; N,5.08.

4a: Yield:76%; mp: 196°C; ^{1}H NMR($CDCl_3$) δ : 1.39 (t,3H,J=7.3 Hz,CH_3), 4.35 (q ,2H,J=7.3 Hz,CH_2), 6.75 (brs,2H,NH_2), 6.98 (s,1H,CH),7.16-7.20(t,2H,Ar-H), 7.84-7.90 (m,2H,Ar-H); IR(KBr): 1660(CO), 1700 (CO), 3360 & 3470 cm^{-1} (NH_2); MS m/z: [M^+] 333(100); Anal. calcd. for $C_{16}H_{12}FNO_4S$: C,57.65; H,3.63; N,4.20. Found: C,57.71; H,3.65; N,4.26.

4b: Yield: 82%; mp: 205°C; ^{1}H NMR(DMSO-d_6) δ : 1.38 (t,3H,J=7.2 Hz,CH_3), 4.24(q,2H,J=7.2 Hz,CH_2), 6.89(s,2H,NH_2), 7.62(d,2H,J=8.1Hz,Ar-H), 7.8(s,1H,CH), 7.92(d,2H,J=8.3 Hz, Ar-H); IR(KBr): 1660 (CO), 1710 (CO), 3380 & 3490 cm^{-1} (NH_2); MS m/z: [M^+] 349 (100); Anal. calcd. for $C_{16}H_{12}ClNO_4S$: C,54.94; H,3.46; N,4.01. Found: C,55.03; H,3.53; N,4.09.

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